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Chapter

Methods for Searching of Potential Beneficial Bacteria and Their Products in Dental Biofilm

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Abstract

Dental microbiota is associated with different types of organisms with dentition including humans and is responsible for many oral diseases all over the world. Bacteria in a dental biofilm are important also in other diseases, i.e., endocarditis, pulmonary fibrosis, and arthritis, and some findings predict the connection of dental microbiota with cancerogenesis. Not all oral bacterial representatives are pathogenic or potentially pathogenic. Dental biofilm consists of numerous different bacteria that may have beneficial characteristics for good condition of dental and oral health. Searching for bacteria or their products with the beneficial effect is important in the development of new biologically based strategies for the prevention or treatment of oral and dental diseases. For searching of potential probiotic candidates are useful methods that could map phenotypic or genotypic characteristics of studied bacteria. This chapter is focused on the spectrum of these basic methods searching for beneficial bacteria and their products.

Keywords: dental, biofilms, probiotic, bacteria, methods

1. Introduction

Each form of life on earth needs to obtain water and some substances from the external environment for its growth. From viruses to whales, every form of life needs some substances. Differences are only in the mechanism of obtaining. Many types of organisms on earth for this purpose developed the digestive tract with the oral cavity during the evolution. The same mechanism is still on earth millions and millions of years. For example, dinosaurs had the same mechanism and during evolution developed dentition for good mechanical preparing of eaten food like humans today with some differences of course. We can deduct, that the dental problems in Jurassic age had the same cause as today if we are thinking about mechanical destruction. In the case of special dental diseases, like periodontitis or dental caries, the comparing is debatable. Maybe in Jurassic age were also some pathogens something like *Streptococcus mutans* nowadays, which were responsible for the destruction of dental enamel in Tyrannosaurus rex. Nowadays problems in dental diseases have the same causative mechanism. Many of them are caused by the effect of dental biofilm bacteria.

1.1 Biofilm, dental biofilm

A biofilm comprises any syntrophic consortium of microorganisms in which cells stick to each other and often also to a surface. Biofilms are highly organized bacterial agglomeration, which diversity is depending on the external and internal conditions of together growing bacteria.

Bacterial biofilms are also characteristic of the growth of one type of bacteria, i.e. a biofilm of *Staphylococcus aureus* [1]. Biofilms may form on living or non-living surfaces and can be founded in natural, industrial, and hospital conditions. In humans, a typical exam for biofilm is dental plaque. This microcosm was deeply characterized with the help of numerous basic or sophisticated methods of research. Microbiology procedures, microscopic techniques, genomic and proteomic methods bring new light on new findings in dental plaque (biofilm) research.

It is interesting, that the knowledge about dental biofilm from the discoveries of Anton van Leeuwenhoek (1632–1723) to today age is still not perfect because we are not able to decrease the number of dental diseases in the world [2].

Dental caries and periodontal diseases are the most common diseases in the world especially in areas with bad quality of dental medicine and in poor regions of the world. On the other side, it is also a disease, which is a wide range presented in all countries and all social communities.

Bacterial pathogens founded in dental enamel lesions are many times highly pathogenic and cause also systematic diseases like endocarditis, meningitis, pulmonary fibrosis, arthritis, and some findings predict the connection of dental microbiota with cancerogenesis [3, 4].

1.2 Dental biofilm bacterial composition

Opinions on the number of bacteria living in the oral cavity vary. It has been estimated that about 500 species of bacteria inhabit the oral cavity in humans [5]. Molecular-based studies have shown that bacterial communities found in the oral cavity are highly complex with about 1000 species and have been shown to be the second most complex microbial community in the body after the colon [6]. Although the animal microbiocenosis of animals and humans has similar properties, there are also significant differences in relation to the microbial species and the relative proportions of these species in the oral cavity [7]. For example, rodents lack gender representatives *Peptostreptococcus, Bacteroides* (currently *Prevotella* and *Porphyromonas*), *Treponema*, *Vibrio* and *Leptotrichia* [8]. Oral microbiocenosis of dogs is believed to be more diverse than oral microbiocenosis in humans [9]. However, bacteria in dental biofilm that are responsible for periodontal infectious diseases in humans and animals have been shown to be similar [7].

The microbiota of the dental biofilm differs from the microbiota on the mucosal surfaces and the composition of the microbiota of the dental biofilm varies in different anatomical sites. Gingival crevice supplies nutrients to bacteria and has low redox potential; therefore, it is colonized predominantly by anaerobic species such as *Prevotella* spp., *Veillonella* spp. and *Fusobacterium* spp. In contrast, supragingival plaque consists mainly of Gram-positive facultatively anaerobic bacteria, especially *Streptococcus* spp. and *Actinomyces* spp. The composition of the oral microbiota is highly dependent on the clinical condition of the teeth and gingivae. Healthy oral plaque contains predominantly facultatively anaerobic Gram-positive species, while in periodontal diseases microbiota turns into obligate anaerobic Gram-negative species [10]. In the formation of dental biofilm, primarily Gram-positive cocci, especially

Streptococcus sanguis and *Streptococcus mitis*, are involved in primary colonization, which colonizes the teeth for the first 4 hours after professional cleansing [11]. Other early colonizers include *Actinomyces* spp., *Capnocytophaga* spp., *Eikenella* spp., *Haemophilus* spp., *Prevotella* spp., *Propionibacterium* spp., and *Veillonella* spp. [12]. One of the major bacteria that serve as a bridge between early and late oral biofilm colonizers is *Fusobacterium nucleatum* [13]. Although it is an anaerobic bacterium, it could tolerate oxygen in the biofilm. This ability allows *F. nucleatum* to promote the growth of other strictly anaerobic bacteria such as *Porphyromonas gingivalis* [14]. Later colonizers are *Lactobacillus* spp., *Porphyromonas* spp., *Actinobacillus* spp., *Prevotella* spp., *Eubacterium* spp., *Selenomonas* spp., *Tannerella* spp., *Aggregatibacter* and *Treponema* spp. [15, 16]. Which type of bacteria, pathogens or potential beneficial members of dental microbiota will be chosen for research depends on the researcher. Currently, preparations containing probiotic strains such as: *Lactobacillus reuteri* (BioGaia Prodentis), *Bacillus coagulans* (Life Extension Advanced Oral Hygiene) and *Streptococcus salivarius* K12 (Bactoral) are available on the market [17–20]. *Bacillus subtilis* in form of tablet [VITALREXTM (VL)] is also used in the treatment of periodontal diseases [21]. Depending on the findings of the beneficial effect of living bacteria, there is also the possibility to use only their metabolic products for research aimed at preventing and treating dental diseases.

2. Recommended methods

2.1 Selection criteria useful for studying of dental biofilm and sample obtaining

In the oral cavity area, it is possible to study apart from dental biofilm also other biofilms, i.e. buccal, lingual, prosthesis, filled live or death teeth, soft tissue biofilms, etc. Our preferred place for obtaining of dental biofilm samples are sites of tooth surfaces close to the salivary duct orifices, because proteins produced in saliva could help to form biofilm and calculus. In humans, it is the lingual surface of the lower front teeth and decreases towards the third molar teeth. On the upper jaw, the supragingival calculus is often formed on the buccal surfaces of the first molars [22]. Also, in veterinary patients, supragingival calculus usually accumulates more rapidly and in larger amounts on the buccal surfaces of the upper jaws [23]. Places for sampling are variable depending on the anatomical proportion of hosts that are used for research as volunteers. Except for humans, it is possible to study dental biofilm also on domesticated or wild animals. Important criteria in the case of human biofilm are smoke, veganism, celiac disease, age, health condition, therapy with medication and so on. Each external and internal factor could change the composition of biofilm and each human has individual microbiota in the mouth. It is better when the group of volunteers has similar dental care (a type of toothpaste used) and similar food consumption habits. The selection of volunteers should be based on the targeted microbiota from the dental biofilm e.g. autochthonous or allochthonous or obtaining of pathogenic bacteria from target pathological lesions in the oral cavity, e.g. caries, etc. Autochthonous microbiota is isolated from volunteers who starve overnight after carefully brushing their teeth. The dental biofilm sample has to be obtained immediately after waking up. Volunteers could not eat, drink or brush their teeth before sampling. The composition of autochthonous or allochthonous microbiota depends on sampling time. If sampling takes place during the day, samples also contain allochthonous microbiota. Better condition for obtaining samples of autochthonous microbiota is from volunteers, which several days do not brush the their teeth.

2.2 Taking of dental biofilm samples

Samples of dental biofilms are easy to obtain, sampling is very simple, painless and noninvasive. Each human volunteer should confirm it with the signed agreement with taking samples, their next processing and provide the data in the anamnestic questionnaire concerning GDPR. In the case of domestic animals, dog or cat, owners have to agree with the possibility of sample taking and processing.

All things that are needed for the researcher are a sterile syringe needle and a sterile Eppendorf tube filled with sterile filtrated PBS commercial produced or according https://www.protocolsonline.com/recipes/phosphate-buffered-saline-pbs/. Cultivation liquid medium can be use for this purpose too.

We provide Brain hearth infusion broth (Merck K GaA Darmstadt, Germany). In the case of lactic acid bacteria isolation, we use deMan, Rogosa and Sharpe MRS (CONDA S.A, Madrid Spain) broth. The blood agar (Tryptic soy agar (TSA)) with 5% ram's blood (BBL, Microbiology Systems, Cockeysville, USA) is often chosen as the first medium for the cultivation of bacteria in bacteriology. In case of selection of major streptococcal species it is good to use Mitis Salivarius Agar (Merck K GaA Darmstadt, Germany). The classical cultivation method is at 37.5°C during 24–48 hours under anaerobic or aerobic conditions, depending on target bacterial members of dental biofilm. We provide BD GasPak™ systems (Becton, Dickinson and Company) for anaerobic cultivation. The further selection of strains is according to the cultivation characteristics of selected colonies. Selected strains could be stored in the glycerol stock or Microbank system (Pro Lab Diagnostic). Each isolated strain has to be identified for further analysis. We provide MALDI-TOF mass spectrometry or Blast n analysis of 16S rRNA sequence for identification. The biochemical tests could help with the identification and reveal the characteristics of the tested strain.

2.3 Methods useful for identification of bacterial composition of dental biofilm

For the study of the bacterial community and its composition, it is possible to use numerous methods. At first, it needs to be mentioned the classical microbiology. By classical bacteriology cultivation methods, we could select different types of cultivable bacteria in samples of dental biofilm. For this purpose, we could use different types of media, from liquid to solid, from basic to highly specific and selective media. Different conditions are also used in aerobic and anaerobic cultivation. The most numerous bacterial resident in the dental biofilm has better start line as low representative bacteria. On the other hand, the conditions in a cultivation medium could bring sometimes better conditions for the growth of former less presented bacteria in a tested sample. Due to this problem, it is hard to declare the ratio of different types of cultivable bacteria. Colonies forming units (CFU) method could reveal the approximate ratio of bacteria, but only the cultivable ones. Quantitative real-time PCR is a cultivation-independent perfect toll for declaring of the bacterial composition of cultivable, hard cultivable or uncultivable bacteria in tested sample, but it is limited due to numbers of selected bacterial groups. Amplicon sequencing is a sensitive method that is cultivation independent and good for declaring the composition of all bacterial members in the tested sample and it could quantify the ratio between bacterial groups [24, 25]. This method is cultivation free and principle is based on the amplification of total DNA isolated from the sample and next-generation sequencing (NGS) analysis. Big data obtained after sequencing are analyzed *in silico*.

If we combine the amplicon sequencing method with 16S rRNA identification of selected and isolated bacteria, we obtain perfect strategy and tools for confirmation of identified cultivable and uncultivable bacteria and also their semiquantitative ratio in our sample of dental biofilm.

It is necessary to know the numbers and ratios of bacteria in the sample because it can bring light to physiological or pathological parameters. On the other hand, it is hard to study this topic, due to the different bacterial composition of individual dental biofilms. Many isolated bacteria are autochthonous and host specific, and still found in a dental biofilm of the individuals. Based on these findings we can predict approximately similar conditions.

Cultivation, isolation, identification, and storage of the strains are necessary steps for deep research of pathogens, potential pathogens, and potentially probiotic strains and research of their interaction.

2.4 Classical cultivation necessary step in research

This method is still necessary for valid research of potentially beneficial bacteria and their products in dental biofilm. For testing of potential candidates as probiotic bacteria from dental biofilm at first, we need to isolate and store it by microbiological cultivation techniques. The same goes for pathogenic bacteria. A very important step in bacteriology research is the identification of bacteria. Form of growth, Gram staining, catalase activity, biochemical parameters are helpful in the analysis of solitary bacterial colonies. These methods are in some cases imperfect for the exact identification of bacteria. In comparison with the methods mentioned above, the sequencing of genes coding 16S RNA or other PCR products and next Blast n analysis or MALDI-TOF mass spectrometry identification are more sensitive.

Other growth characteristics as the possibility of growth inhibition of other bacteria are helpful in the selection of candidates with the production of bioactive substances, especially in the case of biosurfactants, bacteriocins, or bacteriocin-like inhibitory substances (BLIS) [26, 27].

The presence of genes coding bioactive substances could be easily detected by PCR, but better is to check the possibility of their production at first. For example, *Streptococcus salivarius* inhibition potential against *Micrococcus luteus* could help unfold the production of salivaricin [28]. *Streptococcus thermophilus* could induce cell lysis of *Pediococcus acidilactici* and reveal the potential of Thermophilin 110 production [29]. Generally, if the presence of bacteriocins is detected in tested potential probiotics by growth inhibition during cultivation with the target organism, the next step with the help of cultivation and proteomic methods is the overlaying gel test. It can detect the mass size of bacteriocins, i.e. in the case of *Lactobacillus plantarum* or *Streptococcus thermophilus* or other tested bacteria [29, 30]. Cultivation is also necessary to obtain a large volume of bacteriocin for further testing with the help of large volume fermentation. If bacteriocins are soluble and are produced in cultivation media, flow centrifuge is needed for their isolation. If bacteriocins are insoluble it is necessary to use ultracentrifuge in this step. The extraction of bacteriocins is an important step in isolation [31]. If we have pure bacteriocins for testing, cultivation is still needed. For example, salivaricin isolated from *Streptococcus salivarius* K12 is active against bacterial species involved in halitosis, by inhibition of *Micrococcus luteus* Il, *Streptococcus anginosus* T29, *Eubacterium saburreum* ATCC 33271 and *Micromonas micros* ATCC 33270 [28].

Cultivation procedures are needed in case of studying of the capability of the other bioactive substances like biosurfactants or exopolysaccharides. These two products have antagonist effects. Exopolysaccharides enhance adherence and biosurfactants promote disruption of adherence.

It was detected that biosurfactant produced by *Lactobacillus reuteri* could very significantly down-regulate expression of *Streptococcus mutans* glucosyltransferase genes (*gtfB, gtfC*) and fructosyltransferase gene *ftf* [32]. These genes are very important for the production of exopolysaccharides which are responsible for

adherence of oral streptococci [33, 34]. In the case of *Streptococcus mutans* glucosyltransferase genes are responsible for cariogenic activity [35]. These genes are also useful for the differentiation of streptococcal candidates which are often difficult to differentiate because they have high homologous sequences in the 16S rRNA gene.

3. Possibility to produce bioactive substances detected in tested potential bacterial probiotic candidates by PCR (bacteriocins, biosurfactants, and exopolysaccharides)

3.1 Recommended isolation of DNA

The isolation of DNA from bacteria that are difficult to isolate, i.e. lactobacilli strains, is performed by the NucleoSpin® Tissue kit (Macherey-Nagel GmbH and Co. KG, Düren, Germany) using a lysis solution during overnight incubation at 95°C. The next steps of DNA isolation are according to the manufacturer's procedure. It is possible to use other kits for DNA isolation. It depends on researcher choice and routine practice in PCR laboratory. After isolation of DNA it is better to verify DNA quality and quantity. We use Nanodrop spectrophotometric (Wilmington, Delaware USA) analysis for this purpose.

For quick isolation of DNA it is also possible to use one bacterial colony and 100 μl DNAzol direct (Molecular research centre Inc. Cincinnati. USA), and heat it to 95°C during 15 min for isolation of DNA without measuring of DNA quantity, but storage of DNA samples for next analysis is time limited. For storage of DNA isolated by both methods we recommended −20°C. The isolation steps are according to the manufacturer and specific sample.

For PCR we could use Mastermix: One Taq 2X Master Mix (England Biolabs, Ipswich, Massachusetts, USA) and specific primers (**Tables 1–3**) in concentration of 33 μmol at volume 0.6 and 1–2 μl of DNA isolated with help of DNAzol direct.

3.2 Bacteriocins and methods for their detection

A large number of lactic acid bacteria produce bacteriocins that kill other microorganisms. Lactobacilli bacteriocins have potential utility as pathogen inhibitors in humans [36]. Also, oral streptococci have their bacteriocins for example *Streptococcus mutans* have mutacin, and *Streptococcus salivarius* has salivaricin [37, 38]. There are a number of factors influencing the efficacy of bacteriocins *in vivo* and *in situ*, including the survival of the production strain, specific activity, and animal model and targeted pathogen. However, bacteriocins have a great deal of promise to manage various infections and may become an alternative to existing antibiotics. Bacteriocins will need to undergo the same rigorous, costly research and validation process as all other previously approved therapies used in therapy [26]. Recommended conditions for detection of genes coding bacteriocins of some oral potential beneficial bacteria by PCR are described in (**Table 1**).

The researcher could study probiotic or pathogenic bacteria depending of the particular relationship to diseases. For example, PCR condition for bacteriocin detection from *Lactobacillus* spp. is mentioned in the publication [46]. Detection of genes coding production of bacteriocins is only the start of the research. By this method, we could select potential candidates for further research. Inhibition potential can be detected by preferred sensitive bacterial strain for example like in case of *Streptococcus salivarius* salivaricin the sensitive strain is *Micrococcus luteus* [28]. After confirmation of bacteriocin gene presence in tested isolates, there is still much work to be done with purification, fractionation, and isolation of bacteriocins. Not

Target gene	Primers	PCR protocol	Product size	Source
Streptococcus salivarius Salivaricin *sal* A	SalAUS 5′GTAGAAAATATTTACTACATACT3′ SalADS 5′GTTAAAGTATTCGTAAAACTGATG3′	95°C, 13 min, 30× (95°C, 30 sec, 55°C, 1 min, 72°C, 1 min) 72°C, 5 min	338 bp	[38–40]
Lactobacillus reuteri glycerol dehydrogenase *gldC* (reuterin)	GD1f 5′GTTCAGTCCGCCGCATATC3′ GD1r 5′GCCGCTCTTCGTGGATTTC3′	94°C, 5 min, 34× (94°C, 1 min, 58°C, 30 sec, 72°C, 50 sec) 72°C, 7 min	562 bp	[41]
Lactobacillus plantarum Plantaricin	plaF 5′-GGCATAGTTAAAATTCCCCCC-3′ plaR 5′-CAGGTTGCCGCAAAAAAAG-3′	94°C, 5 min, 30× (94°C 45 sec, 53.2°C, 45 sec, 72°C, 45 sec) 72°C, 5 min	428 bp	[42]
Lactobacillus plantarum Plantaricin S	plnF 5′-GCCTTACCAGCGTAATGCCC-3′ plnR 5′-CTGGTGATGCAATCGTTAGTTT-3′	94°C, 5 min, 30× (94°C, 45 sec, 62.3°C, 30 sec, 68°C, 2 min sec) 68°C, 5 min	475 bp	[43]
Streptococcus mutans Mutacin	F 5′-AGTTTCAATAGTTACTGTTGC-3′ R 5′-GCCAAACGGAGTTGATCTCGT-3′	94°C, 5 min, 34× (94°C, 1 min, 58°C, 30 sec, 72°C, 50 sec) 72°C, 7 min	750/450 bp	[44]
Bacillus subtilis Subtilisin	spaSFwd 5′CAAAGTTCGATGATTTCGATTTGGATGT3′ spaSRev 5′GCAGTTACAAGTTAGTGTTTGAAGGAA3′	94°C, 5 min, 34× (94°C, 30 sec, 55°C, 30 sec, 65°C, 60 sec) 65°C, 7 min	722 bp	[45]
Bacillus subtilis Subtilosin	sboAFwd 5′CGCGCAAGTAGTCGATTTCTAACA3′ sboARev R 5′CGCGCAAGTAGTCGATTTCTAACA3′	94°C, 5 min, 34× (94°C, 30 sec, 50°C, 30 sec, 65°C, 60 sec) 65°C, 7 min	565 bp	[45]

Table 1.
PCR conditions used for the detection of gene coding production of bacteriocins.

Target gene	Primers	PCR protocol	Product size	Source
Bacillus subtilis surfactin sfp	sfp F 5′ATGAAGATTTACGGAATTTA3′ sfp R 5′TTATAAAAGCTCTTCGTACG3′	95°C, 3 min, 30× (95°C, 30 sec, 50°C, 30 sec, 72°C, 45 sec) 72°C, 10 min	675	[55]
Bacillus subtilis surfactin srfAA	*srfAA* F 5′TCGGGACAGGAAGACATCAT3′ *srfAA* R 5′CCACTCAAACGGATAATCCTGA3′	95°C, 3 min, 30× (95°C, 30 sec, 60°C, 30 sec, 72°C, 30 sec) 72°C, 10 min	201	[55]
Bacillus subtilis fengycin *fenB*	*fenB* F 5′CCTGGAGAAAGAATATACCGTACCY3′ *fenB* R 5′GCTGGTTCAGTT KGATCACAT3′	95°C, 3 min, 30× (95°C, 30 sec, 57°C, 30 sec, 72°C, 45 sec) 72°C, 10 min	201	[55]
Bacillus subtilis fengycin *fenD*	*fenD* R 5′GCTGGTTCAGTT KGATCACAT3′ *fenD* F 5′GGCCCGTTCTCTAAATCCAT3′	95°C, 3 min, 30× (95°C, 30 sec, 60°C, 30 sec, 72°C, 1 min) 72°C, 5 min	670	[55]
Bacillus subtilis iturín *ituD*	*ituD* F 5′TTGAAYGTCAGYGCSCCTTT3′ *ituD* R 5′TGCGMAAATAATGGSGTCGT3′	95°C, 3 min, 30× (95°C, 30 sec, 57°C, 30 sec, 72°C, 32 sec) 72°C, 10 min	482	[55]
Bacillus subtilis iturín *ituC*	*ituC* F 5′GGCTGCTGCAGATGCTTTAT3′ *ituC* R 5′TCGCAGATAATCGCAGTGAG3′	95°C, 3 min, 30× (95°C, 30 sec, 58°C, 30 sec, 72°C, 30 sec) 72°C, 10 min	423	[55]

Table 2.
PCR conditions used for the detection of gene coding production of biosurfactants.

all bacteria which present genes for bacteriocins are also capable inhibit pathogens. Some inhibition effects are caused by bacteriocins like inhibitory substances or by others active molecules which are waiting to discovered.

3.3 Biosurfactants and methods for their detection

Biosurfactants are naturally produced molecules that demonstrate potentially useful properties such as the ability to reduce surface tensions between different phases [47]. The release of biosurfactants by adhering microorganisms as a defense mechanism against other colonizing strains on the same substratum surface has been described previously for probiotic bacteria in the urogenital tract, the intestines, and the oropharynx, but not for microorganisms in the oral cavity [48]. The antimicrobial properties observed in dialyzed biosurfactants produced by the tested lactobacilli open possibilities for their use against microorganisms responsible for oral diseases [49]. Biosurfactants (BS) obtained from *Lactobacillus* spp. exhibit antibiofilm and antiadhesive activity against a broad spectrum of microbes [50]. For example, they are active against biofilm formation of *Candida albicans* [51] or *Staphylococcus aureus* [52]. Biosurfactants produced by the *Bacillus subtilis* SPB1 strain (HQ392822) revealed a wide spectrum of actions including antimicrobial activity towards multidrug-resistant microorganisms [53, 54]. For the detection of biosurfactants production, i.e. in the case of *Bacillus subtilis*, it is recommended to use PCR with the help of specific primers listed in **Table 2**.

Target gene	Primers	PCR protocol	Product size	Source
Str. mutans. Glucosyltransferase gene (*gtf*)	MKD-F 5′GGCACCACAACATTGGGAAGCTCAGTT3′ MKD-R 5′GGAATGGCCGCTAAGTCAACAGGAT3′	95°C, 13 min, 30× (95°C, 30 sec, 67°C, 1 min, 72°C, 1 min) 72°C, 5 min	433 bp	[60, 61]
Str. salivarius. Glucosyltransferase gene (*gtf*)	MKK-F 5′GTGTTGCCACATCTTCACTCGCTTCG3′ MKK-R 5′CGTTGATGTGCTTGAAAGGGCACCATT3′	95°C, 13 min, 30× (95°C, 30 sec, 66°C, 1 min, 72°C, 1 min) 72°C, 5 min	544 bp	[60]
Str. oralis. Glucosyltransferase gene (*gtf*)	*gtfR* MKR-F 5′TCCCGGTCAGCAAACTCCAGCC3′ *gtfR* MKR-R 5′GCAACCTTTGGATTTGCAAC3′	95°C, 13 min 30× (95°C, 30 sec, 66°C, 1 min, 72°C, 1 min) 72°C, 5 min	374 bp	[60]
Lactobacillus spp. Glucosyltransferase gene (*gtf*)	DexreuV 5′GTGAAGGTAACTATGTTG3′ DexreuR 5′ATCCGCATTAAAGAATGG3′	94°C, 5 min, 31× (94°C, 1 min, 47°C, 1 min, 72°C, 1 min) 72°C, 10 min	600 bp	[62]

Table 3.
PCR conditions used for the detection of gene coding production of exopolysaccharides.

Other species producing biosurfactants and condition for their detection are able in research papers for example: *Lactobacillus paracasei* produced biosurfactants with anti-adhesive properties [56]. *Streptococcus mitis* biosurfactants plays a protective role in the oral cavity and protects against colonization of saliva-coated surfaces by cariogenic *Streptococcus mutans* [48]. Based on *Bacillus subtilis* SPB1 lipopeptides production researcher predict their possibility used in toothpaste formulation [53]. Biosurfactants are promising bioactive molecules for oral-related health applications [47].

3.4 Exopolysaccharides and methods for their detection

Lactic acid bacteria are the most frequently mentioned in studies of exopolysaccharides (EPS) in oral microbiota [57]. Except for lactobacilli, which are participated in the later stages of dental biofilm formation, streptococci are one of the first bacteria capable of producing EPS. Streptococci are able to assert themselves and adhere to the hard tissues of the oral cavity immediately after washing the teeth. This property of adherence is predetermined and is encoded in genes that are also responsible for the production of glucosyltransferases. Glucosyltransferases (Gtfs) are produced by several types of lactic acid bacteria [58]. Gtfs are generally characterized as Gtf-S (glucosyltransferase-soluble) or Gtf-I (glucosyltransferase-insoluble) enzymes, depending on whether the glucan they produce is water soluble or insoluble [59]. For detection of exopolysaccharides production in oral lactic acid bacterial members is useful PCR with help of specific primers see in **Table 3**.

4. Testing of growth inhibition activity against pathogens

Testing of bacterial isolates as potential beneficial candidates or their products is necessary step in new discoveries. We are able declarate production of bioactive substance by very easy PCR reactions, as mentioned above in part 3. Activity of these substances is easy to declare by simply *in vitro* tests. At first for activity it is possible to use spot on or disc diffusion test. Same mechanism of declaration is for live bacteria isolates as for isolated bioactive substances.

If we found bacteria with interesting effect in spot or disc diffusion test it predict selection criteria of former characterized bacteria for next research.

4.1 The disc diffusion method for *Lactobacillus reuteri* for testing of growth inhibition activity against pathogens

We recommend the disc diffusion test for the detection of the inhibitory properties of beneficial microorganisms. Selected lactobacilli strains were grown on MRS agar (CONDA S.A, Madrid Spain) for 48 hours. anaerobically (Gas Pak Plus, BBL, Microbiology Systems, Cockeysville, USA) at 37°C. Then, a standardized suspension with an optical density of 1 McFarland by dissolving several solitary colonies in 5 ml of physiological saline was prepared. Sterile clean discs (6 mm diameter, BBL, Cockeysville, USA) were placed on Petri dishes (Ø 90 mm) with 20 ml of PYG agar (HiMedia Laboratories GmbH Einhausen, Germany). The sterile paper discs were inoculated with 5 μl of standardized suspensions of lactobacilli.

As a negative control, one Petri dish with PYG agar is served with a clean paper discs soaked with sterile MRS broth.

The plates with discs were incubated for 48 hours. anaerobically (Gas Pak Plus, BBL, Microbiology Systems, Cockeysville, USA) at 37°C. The discs were removed with a sterile syringe needle or tweezer after incubation. Subsequently, 3 ml of 0.7% PYG agar was inoculated with 0.3 ml of the indicator pathogenic strain and put into

plates with lactobacilli. Pathogenic strains were incubated for 18 hours in PYG broth at 37°C. The plates with YPG medium inoculated with pathogen were incubated for 24 hours aerobically at 37°C. After incubation, the diameter of the inhibition zones was measured. The results were recorded in the table as the arithmetic means of the three measurements ± standard deviation.

4.2 The disc diffusion method for *Streptococcus salivarius* for testing of growth inhibition activity against pathogens

The disc diffusion test with *Micrococcus luteus* was used for the preliminary testing of *Streptococcus salivarius* inhibition [39]. This test analyses the activity of the BLIS produced in agar and determines the activity spectrum of Sal9 producers. Briefly, the tested strain was inoculated across the surface of the Blood agar medium (BBL, Microbiology Systems, Cockeysville, USA) in a glass Petri dish (Ø 90 mm) as a 1 cm-wide streak. After incubation, the strain growth was stopped by its exposure to chloroform vapor for 30 min. The plate was then aired for 15 min before 24 hours inoculating cultures as the indicator strains across the original tested strain. The plate was incubated for 24 hours and examined for the zones of the indicator strain growth inhibition. The inhibition activity against the selected standard indicators was recorded in code form by inoculating the indicators in three triplets. The inhibition of the first member of a triplet was given a score of 4, the second a score of 2, and the third a score of 1. The absence of the inhibitory action against an indicator was scored as 0. The code was recorded as a sequence of three numbers representing the sum of each triplet. All tests were performed in duplicate, and further testing was undertaken until the consistency of the inhibition patterns was obtained [63].

5. Conclusion

It is necessary to know the composition of the dental biofilm of healthy individuals and the bacterial composition in pathological conditions to identify species responsible for disease initiation and progression. Identification of species and their characterization is essential for the selection of pathogenic, potentially pathogenic and potentially probiotic species. Blast n analysis of 16S RNA or MALDI-TOF mass spectrometry identification is perfect tools for identification of bacterial species. The ability to modulate the microbiocenosis of the dental biofilm by bacteria living together in the biofilm should be studied. The some bacteria are capable of producing bioactive substances whose presence we can quickly and easily declare with help of PCR. Sequencing and comparing of genes coding bioactive substances can uncover differences between tested bacteria isolates. Presence of these genes and prove the ability to inhibit the growth of other bacterial species are important steps in selection of potentially probiotic candidates. These bacteria are of great interest for further study and may be useful in the development of new antibacterial agents. Bioactive substances can be extracted by physical methods (centrifugation, separation and fractionation), by chemical methods (purification) and detected by modern analytical method (HPLC) or proteomic methods (MALDI-TOF MS). Next important step is declaration of activity pure extracted substance. Bioactive substances of bacterial origin can be used in dental preparations and serve as prevention or supplementary therapy of periodontal diseases. During recent years there has occurred a shift towards ecological and microbial community based approach to the therapy of oral cavity diseases. With the increasing resistance to antibiotics, the use of probiotics appears as a prospective alternative treatment or preventative measure in the control of periodontal diseases. From the clinical point

of view, it is not yet possible to give direct recommendations for the use of probiotics. However, the available scientific evidence indicates that probiotic therapy is a promising approach also in the field of stomatology. The potential beneficial strains of *Streptococcus salivarius* or *Lactobacillus reuteri* and others bacterial strains isolated from many oral biofilms can be selected for next research based on their production of bioactive substances and on growth inhibition level against oral pathogenic bacteria not only in human but also in social animals like dogs and cats.

Acknowledgements

This publication was supported by the project of the Ministry of education science Research and sport of the Slovak Republic VEGA 1/0788/19: "*Study of changes in microbiota of dental biofilms in humans and dogs in order to harmonize the oral microbiocenosis by using the selected oral probiotics*" and by the Slovak Research and Development Agency under the contract no. APVV-16-0203.

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References

[1] Pontes EKU, Melo HM, Nogueira JWA, et al. Antibiofilm activity of the essential oil of citronella (*Cymbopogon nardus*) and its major component, geraniol, on the bacterial biofilms of *Staphylococcus aureus*. Food Science and Biotechnology. 2019;**28**(3):633-639. DOI: 10.1007/s10068-018-0502-2502

[2] Hicks J, Garcia-Godoy F, Flaitz C. Biological factors in dental caries: Role of remineralization and fluoride in the dynamic process of demineralization and remineralization (part 3). The Journal of Clinical Pediatric Dentistry. 2004;**28**(3):203-214

[3] Debelian GJ, Olsen I, Tronstad L. Systemic diseases caused by oral microorganisms. Endodontics & Dental Traumatology. 1994;**10**(2):57-65

[4] Karpinski TM. Role of Oral microbiota in cancer development. Microorganisms. 2019;**7**(1):20. DOI: 10.3390/microorganisms7010020

[5] Paster BJ, Boches SK, Galvin JL, et al. Bacterial diversity in human subgingival plaque. Journal of Bacteriology. 2001;**183**(12):3770-3783. DOI: 10.1128/JB.183.12.3770-3783.2001

[6] Wade WG. The oral microbiome in health and disease. Pharmacological Research. 2012;**69**(1):137-143. DOI: 10.1016/j.phrs.2012.11.006

[7] Percival S, Knottenbelt D, Cochrane C. Biofilms and Veterinary Medicine. Vol. 6. Berlin Heidelberg: Springer-Verlag; 2011

[8] Elliott DR, Wilson M, Buckley CM, et al. Cultivable oral microbiota of domestic dogs. Journal of Clinical Microbiology. 2005;**43**(11):5470-5476. DOI: 10.1128/jcm.43.11.5470-5476.2005

[9] Oh C, Lee K, Cheong Y, et al. Comparison of the oral microbiomes of canines and their owners using next-generation sequencing. PLoS One. 2015;**10**(7):e0131468. DOI: 10.1371/journal.pone.0131468

[10] Kleessen B, Bezirtzoglou E, Mättö J. Culture-based knowledge on biodiversity, development and stability of human gastrointestinal microflora. Microbial Ecology in Health and Disease. 2000;**12**(Suppl. 2):53-63

[11] Elmar Hellwig JK, Attin T. Záchovná stomatologie a parodontologie. Praha: Grada Publishing; 2003

[12] Kolenbrander PE, Andersen RN, Blehert DS, et al. Communication among oral bacteria. Microbiology and Molecular Biology Reviews. 2002;**66**(3):486-505

[13] Benitez-Paez A, Belda-Ferre P, Simon-Soro A, et al. Microbiota diversity and gene expression dynamics in human oral biofilms. BMC Genomics. 2014;**15**:311. DOI: 10.1186/1471-2164-15-311

[14] Diaz PI, Rogers AH, Zilm PS. *Fusobacterium nucleatum* supports the growth of *Porphyromonas gingivalis* in oxygenated and carbon-dioxide-depleted environments. Microbiology. 2002;**148**(Pt 2):467-472. DOI: 10.1099/00221287-148-2-467

[15] Badet C, Thebaud NB. Ecology of lactobacilli in the oral cavity: A review of literature. The Open Microbiology Journal. 2008;**2**:38-48. DOI: 10.2174/1874285800802010038

[16] Struzycka I. The oral microbiome in dental caries. Polish Journal of Microbiology. 2014;**63**(2):127-135

[17] Stensson M, Koch G, Coric S, et al. Oral administration of *Lactobacillus reuteri* during the first year of life reduces caries prevalence in the primary

dentition at 9 years of age. Caries Research. 2013;**48**(2):111-117. DOI: 10.1159/000354412

[18] Konuray G, Erginkaya Z. Potential use of *Bacillus coagulans* in the food industry. Food. 2018;**7**(6):92. DOI: 10.3390/foods7060092

[19] Ahola AJ, Yli-Knuuttila H, Suomalainen T, et al. Short-term consumption of probiotic-containing cheese and its effect on dental caries risk factors. Archives of Oral Biology. 2002;**47**(11):799-804. DOI: 10.1016/s0003-9969(02)00112-7

[20] Caglar E, Cildir SK, Ergeneli S, et al. Salivary mutans streptococci and lactobacilli levels after ingestion of the probiotic bacterium *Lactobacillus reuteri* ATCC 55730 by straws or tablets. Acta Odontologica Scandinavica. 2006;**64**(5):314-318. DOI: 10.1080/00016350600801709

[21] Tsubura S, Mizunuma H, Ishikawa S, et al. The effect of *Bacillus subtilis* mouth rinsing in patients with periodontitis. European Journal of Clinical Microbiology & Infectious Diseases. 2009;**28**(11):1353-1356. DOI: 10.1007/s10096-009-0790-9

[22] Jin Y, Yip HK. *Supragingival calculus*: Formation and control. Critical Reviews in Oral Biology and Medicine. 2002;**13**(5):426-441

[23] Borah BM, Halter TJ, Xie B, et al. Kinetics of canine dental calculus crystallization: An in vitro study on the influence of inorganic components of canine saliva. Journal of Colloid and Interface Science. 2014;**425**:20-26. DOI: 10.1016/j.jcis.2014.03.029

[24] Rasmussen K, Nikrad J, Reilly C, et al. N-Acetyl-l-cysteine effects on multi-species oral biofilm formation and bacterial ecology. Letters in Applied Microbiology. 2015;**62**(1):30-38. DOI: 10.1111/lam.12513

[25] Anderson AC, Rothballer M, Altenburger MJ, et al. In-vivo shift of the microbiota in oral biofilm in response to frequent sucrose consumption. Scientific Reports. 2018;**8**(1):1-13. DOI: 10.1038/s41598-018-32544-6

[26] Hammami R, Fernandez B, Lacroix C, et al. Anti-infective properties of bacteriocins: An update. Cellular and Molecular Life Sciences. 2012;**70**(16):2947-2967. DOI: 10.1007/s00018-012-1202-3

[27] Plaza G, Chojniak J, Rudnicka K, et al. Detection of biosurfactants in *Bacillus* species: Genes and products identification. Journal of Applied Microbiology. 2015;**119**(4):1023-1034. DOI: 10.1111/jam.12893

[28] Burton JP, Chilcott CN, Wescombe PA, et al. Extended safety data for the oral cavity probiotic *Streptococcus salivarius* K12. Probiotics and Antimicrobial Proteins. 2010;**2**(3):135-144. DOI: 10.1007/s12602-010-9045-4

[29] Gilbreth SE, Somkuti GA. Thermophilin 110: A bacteriocin of *Streptococcus thermophilus* ST110. Current Microbiology. 2005;**51**(3):175-182. DOI: 10.1007/s00284-005-4540-7

[30] Rattanachaikunsopon P, Phumkhachorn P. Isolation and preliminary characterization of a bacteriocin produced by *Lactobacillus plantarum* N014 isolated from nham, a traditional Thai fermented pork. Journal of Food Protection. 2006;**69**(8):1937-1943

[31] Elayaraja S, Annamalai N, Mayavu P, et al. Production, purification and characterization of bacteriocin from *Lactobacillus murinus* AU06 and its broad antibacterial spectrum. Asian Pacific Journal of Tropical Biomedicine. 2014;**4**(1):305-311. DOI: 10.12980/APJTB.4.2014C537apjtb-04-s1-s305

[32] Salehi R, Savabi O, Kazemi M, et al. Effects of *Lactobacillus reuteri*-derived biosurfactant on the gene expression profile of essential adhesion genes (gtfB, gtfC and ftf) of *Streptococcus mutans*. Advanced Biomedical Research. 2014;**3**:169. DOI: 10.4103/2277-9175.139134

[33] Lairson LL, Henrissat B, Davies GJ, et al. Glycosyltransferases: Structures, functions, and mechanisms. Annual Review of Biochemistry. 2008;**77**:521-555. DOI: 10.1146/annurev.biochem.76.061005.092322

[34] Tieking M, Korakli M, Ehrmann MA, et al. In situ production of exopolysaccharides during sourdough fermentation by cereal and intestinal isolates of lactic acid bacteria. Applied and Environmental Microbiology. 2003;**69**(2):945-952. DOI: 10.1128/aem.69.2.945-952.2003

[35] Bowen WH, Koo H. Biology of *Streptococcus mutans*-derived glucosyltransferases: Role in extracellular matrix formation of cariogenic biofilms. Caries Research. 2011;**45**(1):69-86. DOI: 10.1159/000324598

[36] Ruiz FO, Gerbaldo G, Garcia MJ, et al. Synergistic effect between two bacteriocin-like inhibitory substances produced by lactobacilli strains with inhibitory activity for *Streptococcus agalactiae*. Current Microbiology. 2012;**64**(4):349-356. DOI: 10.1007/s00284-011-0077-0

[37] Woodruff WA, Novak J, Caufield PW. Sequence analysis of mutA and mutM genes involved in the biosynthesis of the lantibiotic mutacin II in *Streptococcus mutans*. Gene. 1998;**206**(1):37-43

[38] Wescombe PA, Upton M, Dierksen KP, et al. Production of the lantibiotic salivaricin A and its variants by oral streptococci and use of a specific induction assay to detect their presence in human saliva. Applied and Environmental Microbiology. 2006;**72**(2):1459-1466. DOI: 10.1128/aem.72.2.1459-1466.2006

[39] Barbour A, Philip K, Muniandy S. Enhanced production, purification, characterization and mechanism of action of salivaricin 9 lantibiotic produced by *Streptococcus salivarius* NU10. PLoS One. 2013;**8**(10):e77751. DOI: 10.1371/journal.pone.0077751

[40] O'Shea EF, Gardiner GE, O'Connor PM, et al. Characterization of enterocin- and salivaricin-producing lactic acid bacteria from the mammalian gastrointestinal tract. FEMS Microbiology Letters. 2009;**291**(1):24-34. DOI: 10.1111/j.1574-6968.2008.01427.x

[41] Kinova Sepova H, Bilkova A. Isolation and identification of new lactobacilli from goatling stomach and investigation of reuterin production in *Lactobacillus reuteri* strains. Folia Microbiologia. 2012;**58**(1):33-38. DOI: 10.1007/s12223-012-0166-x

[42] Suwanjinda D, Eames C, Panbangred W. Screening of lactic acid bacteria for bacteriocins by microbiological and PCR methods. Biochemistry and Molecular Biology Education. 2007;**35**(5):364-369. DOI: 10.1002/bmb.84

[43] Stephens SK, Floriano B, Cathcart DP, et al. Molecular analysis of the locus responsible for production of plantaricin S, a two-peptide bacteriocin produced by *Lactobacillus plantarum* LPCO10. Applied and Environmental Microbiology. 1998;**64**(5):1871-1877

[44] Kamiya RU, Napimoga MH, Hofling JF, et al. Frequency of four different mutacin genes in *Streptococcus mutans* genotypes isolated from caries-free and caries-active individuals. Journal of Medical Microbiology. 2005;**54**(6):599-604. DOI: 10.1099/jmm.0.45870-0

[45] Sutyak KE, Wirawan RE, Aroutcheva AA, et al. Isolation of the *Bacillus subtilis* antimicrobial peptide subtilosin from the dairy product-derived *Bacillus amyloliquefaciens*. Journal of Applied Microbiology. 2008;**104**(4):1067-1074. DOI: 10.1111/j.1365-2672.2007.03626.x

[46] Chervinets Y, Chervinets V, Shenderov B, et al. Adaptation and probiotic potential of lactobacilli, isolated from the oral cavity and intestines of healthy people. Probiotics and Antimicrobial Proteins. 2017;**10**(1):22-33. DOI: 10.1007/s12602-017-9348-9

[47] Elshikh M, Marchant R, Banat IM. Biosurfactants: promising bioactive molecules for oral-related health applications. FEMS Microbiology Letters. 2016;**363**(18):1-7, fnw213

[48] van Hoogmoed CG, van Der Kuijl-Booij M, van Der Mei HC, et al. Inhibition of *Streptococcus mutans* NS adhesion to glass with and without a salivary conditioning film by biosurfactant-releasing *Streptococcus mitis* strains. Applied and Environmental Microbiology. 2000;**66**(2):659-663. DOI: 10.1128/aem.66.2.659-663.2000

[49] Ciandrini E, Campana R, Casettari L, et al. Characterization of biosurfactants produced by *Lactobacillus* spp. and their activity against oral streptococci biofilm. Applied Microbiology and Biotechnology. 2016;**100**(15):6767-6777. DOI: 10.1007/s00253-016-7531-7

[50] Satpute SK, Mone NS, Das P, et al. Inhibition of pathogenic bacterial biofilms on PDMS based implants by *L. acidophilus* derived biosurfactant. BMC Microbiology. 2019;**19**(1):39. DOI: 10.1186/s12866-019-1412-z

[51] Cochis A, Fracchia L, Martinotti MG, et al. Biosurfactants prevent in vitro *Candida albicans* biofilm formation on resins and silicon materials for prosthetic devices. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology. 2012;**113**(6):755-761. DOI: 10.1016/j.oooo.2011.11.004

[52] Merghni A, Dallel I, Noumi E, et al. Antioxidant and antiproliferative potential of biosurfactants isolated from *Lactobacillus casei* and their anti-biofilm effect in oral *Staphylococcus aureus* strains. Microbial Pathogenesis. 2017;**104**:84-89. DOI: 10.1016/j.micpath.2017.01.017

[53] Bouassida M, Fourati N, Krichen F, et al. Potential application of *Bacillus subtilis* SPB1 lipopeptides in toothpaste formulation. Journal of Advanced Research. 2017;**8**(4):425-433. DOI: 10.1016/j.jare.2017.04.002

[54] Ghribi D, Abdelkefi-Mesrati L, Mnif I, et al. Investigation of antimicrobial activity and statistical optimization of *Bacillus subtilis* SPB1 biosurfactant production in solid-state fermentation. Journal of Biomedicine & Biotechnology. 2012;**2012**:373682. DOI: 10.1155/2012/373682

[55] Chung S, Kong H, Buyer JS, et al. Isolation and partial characterization of *Bacillus subtilis* ME488 for suppression of soilborne pathogens of cucumber and pepper. Applied Microbiology and Biotechnology. 2008;**80**(1):115-123. DOI: 10.1007/s00253-008-1520-4

[56] Gudina EJ, Teixeira JA, Rodrigues LR. Isolation and functional characterization of a biosurfactant produced by *Lactobacillus paracasei*. Colloids and Surfaces. B, Biointerfaces. 2009;**76**(1):298-304. DOI: 10.1016/j.colsurfb.2009.11.008

[57] Schwab C, Walter J, Tannock GW, et al. Sucrose utilization and impact of sucrose on glycosyltransferase expression in *Lactobacillus reuteri*. Systematic and Applied Microbiology.

2007;**30**(6):433-443. DOI: 10.1016/j.syapm.2007.03.007

[58] Argimon S, Alekseyenko AV, DeSalle R, et al. Phylogenetic analysis of glucosyltransferases and implications for the coevolution of mutans streptococci with their mammalian hosts. PLoS One. 2013;**8**(2):e56305. DOI: 10.1371/journal.pone.0056305

[59] Kingston KB, Allen DM, Jacques NA. Role of the C-terminal YG repeats of the primer-dependent streptococcal glucosyltransferase, GtfJ, in binding to dextran and mutan. Microbiology. 2002;**148**(2):549-558. DOI: 10.1099/00221287-148-2-549

[60] Hoshino T, Kawaguchi M, Shimizu N, et al. PCR detection and identification of oral streptococci in saliva samples using gtf genes. Diagnostic Microbiology and Infectious Disease. 2004;**48**(3):195-199. DOI: 10.1016/j.diagmicrobio.2003.10.002

[61] Al-Ahmad A, Auschill TM, Braun G, et al. Overestimation of *Streptococcus mutans* prevalence by nested PCR detection of the 16S rRNA gene. Journal of Medical Microbiology. 2006;**55**(1):109-113. DOI: 10.1111/j.1365-2672.2005.02638.x

[62] Tieking M, Kaditzky S, Valcheva R, et al. Extracellular homopolysaccharides and oligosaccharides from intestinal lactobacilli. Journal of Applied Microbiology. 2005;**99**(3):692-702. DOI: 10.1111/j.1365-2672.2005.02638.x

[63] Wescombe PA, Upton M, Renault P, et al. Salivaricin 9, a new lantibiotic produced by *Streptococcus salivarius*. Microbiology. 2011;**157**(5):1290-1299. DOI: 10.1099/mic.0.044719-0